Cell Counting Kit-8

Catalog No.: CSK1160

Kit Content

CCK-8 solution 5ml

Storage

Store at 4℃ in dark for one year

Introduction

Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing Dojindo's highly water-soluble tetrazolium salt. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, monosodium salt]* produces a water-soluble formazan Dye upon reduction in the presence of an electron carrier,as shown in Figure 1.Cell Counting Kit-8 is a one-bottle solution; no premixing of components is required. Cell Counting Kit-8, being nonradioactive, allow sensitive colorimetric assays for the determination of the number of viable cells n cell proliferation and cytotoxicity assays. WST-8 is educed by dehydrogenases in cells to give a yellowcolored roduct (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. The detection sensitivity of CCK-8 is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1.

The kit components are sufficient for performing up to 500 assays.

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Protocol

1. Collect logarithmic phase cell, adjust cell suspension concentration; add 100ul floor plate. In general, cells seeded at densities between 1000-10,000 cells per well (side holes filled with aseptic PBS buffer).
2. Seed cells in a 5% Co2 incubator at 37℃ until cells bespread well bottom for one floor (cells number for each well is according to cells' size and breed speed). Add concentration gradient drug. Principlely, add drug after cells adhere. 0-10ul per well. Using 3-5 repeating pipettors.
3. Add 10μl CCK-8 into each well.(considering the ratio 1:10). Choose the wells without cells as contrast wells.
4. Incubate for 0.5-4 hours, usually 1 hour is enough. The incubate time response to the situation of cell's type and concentration. You can try to read the result after 0.5 hour, 1hour, 2 hours and 4 hours solely at first time, then chose a proper time for next step.
5. Read absorbance at 450nm. If there's no 450nm filter, use 420-480nm instead. During dual wavelength spectrophotometry, you may choose wavelength longer than 600nm.

Note

1. If cell culture time is too long, please pay attention to the evaporation issue. Avoid using the outmost wells, add PBS buffer, water or culture fluid instead; or place 96 wells near by the water in incubator.
2. This kit bases on the catalytic reaction of dehydrogenates. If there are too many reducing agents in the system ready to detect, such as some antioxidant which may interrupt the result, you should remove them first.
3. Make sure that there's no bubble in any well before use the ELISA reader, or it will interrupt the result.
4. Please wear transparent gloves when operate.